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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Comparison of Secondary Metabolites and Essential Oil Content of Some *Origanum* Species

Bazı Origanum Türlerinin Sekonder Metabolitleri ve Uçucu Yağ İçeriklerinin Karşılaştırılması

Muhsin AĞAMİRZAOĞLU[*1], Negar VALIZADEH[2], Amir RAHIMI[3]

Abstract

Origanum genus is one of the most widely used herbs in folk medicine for its biological properties. This study was performed to determine the morphological and phytochemical properties of five species of *Origanum* including *O. majorana*, *O. onites*, *O. syriacum*, *O. vulgare* subsp. *vulgare* and *O. vulgare* subsp. *hirtum* as important ethnomedicinal plants. The study was performed at a Research Farm based at Urmia University, Iran. The plants were collected from various places for determining some quantitative properties, antioxidant compounds, and essential contents. The results showed that the highest plant height (86.4 cm) was observed in *O. vulgare* subsp. *hirtum*. The highest fresh weight and dry weight were observed in *O. onites* (826 and 250 g) and *O. vulgare* subsp. *hirtum* (727.64 and 230 g) species in comparison to others, respectively. However, the highest essential oil, essential oil yield per plant, and essential oil yield per ha were 5.26%, 1.71 g and 114 kg ha^{-1}, respectively, which was observed in *O. vulgare* subsp. *hirtum* species. The quantitative analysis revealed higher content of total phenol (51.12%), flavonoid (6.93%), 2, 2-diphenyl-1-picrylhydrazyl (DPPH), (54.29%), superoxide (50.04%) and radical scavenging activities in *O. vulgare* subsp. *hirtum* species, but the *O. onites* species showed higher (21.60%) nitric oxide radical scavenging activities compared to other species. The essential oil analysis revealed that the thymol (6.90-59.89%), carvacrol (0.83-48.91%), γ-terpinene (6.55-18.20%), p-cymene (0.50-20.94%) and α-terpinene (2.71-4.28%) were the most prominent compounds in the studied species of the genus *Origanum*. Cluster analysis showed two main categories and high similarity between *O. onites* and *O. vulgare* subsp. *hirtum*. The findings of the current research indicate that *O. vulgare* subsp. *hirtum* was the best species in terms of phytochemical properties.

Keywords: Marjoram, Phytochemicals, Secondary metabolites, Thymol

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Öz

Origanum cinsi, biyolojik özelliklerinden dolayı geleneksel tıpta en çok kullanılan şifalı bitkilerden biridir. Bu çalışma, beş *Origanum* türünün, *O. majorana*, *O. onites*, *O. Syriacum*, *O. vulgare* subsp. *vulgare* ve *O. vulgare* subsp *hirtum* morfolojik ve fitokimyasal özelliklerini belirlemeyi amaçlamaktadır. Çalışma, İran Urmiye Üniversitesi araştırma alanında yürütülmüştür. Bitkiler bazı kantitatif özellikleri, antioksidan bileşikleri ve uçucu yağ içeriklerini belirlemek için çeşitli yerlerden toplanmıştır. Sonuçlara göre en yüksek bitki boyu (86.4 cm) *O. vulgare* subsp. *hirtum* gözlenmiştir. En yüksek taze bitki ağırlığı ve kuru ağırlığı diğer türler ile karşılaştırıldığında *O. onites* (826 ve 250 g) ve *O. vulgare* subsp. *hirtum* (727.64 ve 230 g) türlerinde görülmüştür. Fakat en yüksek uçucu yağ, bitki başına uçucu yağ verimi ve hektar başına uçucu yağ verimi sırasıyla %5.26, 1.71 g ve 114 kg/ha ile *O. vulgare* subsp *hirtum* türünde gözlenmiştir. Kantitatif analiz sonuçlarına göre; en yüksek toplam fenol içeriği (%51.12), flavonoid (%6.93), 2, 2-difenil-1-pikrilhidrazil (DPPH), (%54.29), süperoksit (%50.04) içeriği ve radikal yakalama aktiviteleri *O. vulgare* subsp. *hirtum*'da ortaya konmuştur, ancak diğer türlerle karşılaştırıldığında, *O. onites* daha yüksek (%21.60) nitrik oksit radikali yakalama aktiviteleri göstermiştir. Uçucu yağ analizleri, Origanum cinsinin incelenen türlerinde, timol (%6.90-59.89), karvakrol (%0.83-48.91), γ-terpinen (%6.18-55.20), p-simen (%94 0.50-0.20) ve a-terpinenin (%2.71-) temel bileşenler olduğunu göstermiştir. Küme analizi, *O. onites* ve *O. vulgare* subsp *hirtum* arasında iki ana kategori ve yüksek benzerlik göstermektedir. Bu araştırmanın bulgularına göre fitokimyasal özellikler açısından *O. vulgare* subsp *hirtum*'un en iyi tür olduğu belirlenmiştir.

Anahtar Kelimeler: Mercanköşk; Fitokimyasallar; Sekonder Metabolitler; Timol

1. Introduction

Medicinal plants are sources of various secondary metabolites with high pharmaceutical activities (Pandey et al., 2019; Mirzapour et al., 2022; Rahimi et al., 2022; Faridvand et al., 2021; Salvo et al., 2018). Secondary metabolites have high added value depending on their production and supply. The value of raw medicinal herbs and their products is significantly growing in the world markets (Pezzani et al., 2017; Tripathy et al., 2017; Rahimi et al., 2022), with ever increase in their demand and selling (Bhardwaj et al., 2019; Ramakrishna et al., 2019) which emphasis on replacing chemically produced medicines with natural medicines (Duletić-Laušević et al., 2018; Morshedloo et al., 2018; Aktepe et al., 2019). Many flowering and aromatic plant species in the *Lamiaceae* family, that are distributed worldwide, are mainly cultivated for their fragrant leaves and attractive flowers (Amiri et al., 2018; Méabed et al., 2018; Moghrovyan et al., 2019; Pandey et al., 2019; Raudone et al., 2018). The species in the genus are highly valued due to the presence of aromatic compounds present in the external glandular structures that produce volatile oils (Ramakrishna et al., 2019; Spyridopoulou et al 2019). These species are widely utilized as pharmaceutical and flavoring herbs around the world and include biologically active elements such as phenolic glucosides, flavonoids, resins, tannins, terpenoids, and sterols (Pezzani et al., 2017; Tripathy et al., 2017). According to Salvo et al. (2018) and Ramakrishna et al. (2019), carvacrol in *Origanum* essential oil presumably interacts with the release and/or production of inflammatory mediator molecules including prostanoid, hence improving the process of healing stomach ulcers.

Some researchers have demonstrated that oregano essential oil has antioxidant, antibacterial, antifungal, and antimicrobial effects, as well as the ability to treat gastrointestinal ailments (Elshamy et al., 2018; Mirzapour et al., 2022; Marrelli et al., 2018). This explains why such crops are highly sourced by the nourishment, cosmetic, pharmaceutical, and medical industries (Spyridopoulou et al., 2019). These species are also used for seasonings, flavor-enhancing, and increasing the shelf life of foods (Bhardwaj et al., 2019; Koleva et al., 2018) making them very popular in the ethnomedicinal culture of the Middle East including Türkiye (Oke-Altuntas et al., 2018). These plant species are adapted to diverse climates and are frequently utilized in the cosmetics and pharmaceutical industries due to their very diverse aromatic constituents. Medicinal properties of such species are often ascribed to the presence of high contents of volatile compounds. *O. vulgare*, *O. majoran*a, *O. onites*, *O. syriacum* are the most plentiful among the genus *Origanum* (Salvo et al., 2018; Kaplan et al., 2019).

It is possible the pharmaceutical properties and antioxidant effects of different *Origanum* plant species differ from one another (Hassan et al., 2020). Therefore, it is important to explore the detailed phytochemical properties of these plant species for wider utilization. This will encourage their widescale extensive cultivation. Therefore, this study's key objective was to evaluate the secondary metabolites and morphological properties of five *Origanum* species cultivated in Urmia and to encourage their wide range of commercial products based on scientific standards.

2. Materials and Methods

2.1. Study Area

The study was executed in a Research farm based in the Plant Production and Genetics Department, Urmia University, Iran, during the crop years of 2016–2017. It was carried out in randomized blocks, and replicated thrice. The climate data of the research location (Urmia University, Iran), as well as soil analysis results from the site, were recorded. The plant nursery of five *Origanum* species (*O. onites, O.majorana*, *O.syriacum*, *O. vulgare* subsp. *vulgare* and *O. vulgare* subsp. *hirtum* (*Figure 1*) was conducted in a greenhouse. The seeds were planted in wrapped 1-liter plastic pots with equally distributed peat moss, sand, and soil. The pots were regularly watered based on greenhouse situations and the phase of plant growth. Once the seed germinated to form nursery seedlings, they were transplanted to the experimental fields in autumn. The experimental ground was plowed at a proper moisture condition of 70-75% using sentek soil moisture probes (capacity of the field) and smoothed. Phosphorus and potassium fertilizers were added before seeding, following the recommendations of the soil analysis report, and furrowed in 50 cm rows. According to the recommendations in the soil analysis report, nitrogen fertilizer was used during the planting and vegetative phases. Watering was carried out at 60% moisture content in the upper (0-30 cm) soil layer. Harvesting of leaves was done at 50% flowering in the second year two times. The harvested plant leaves were dehydrated at room temperature until they attained a constant 8-10% moisture level by weight.

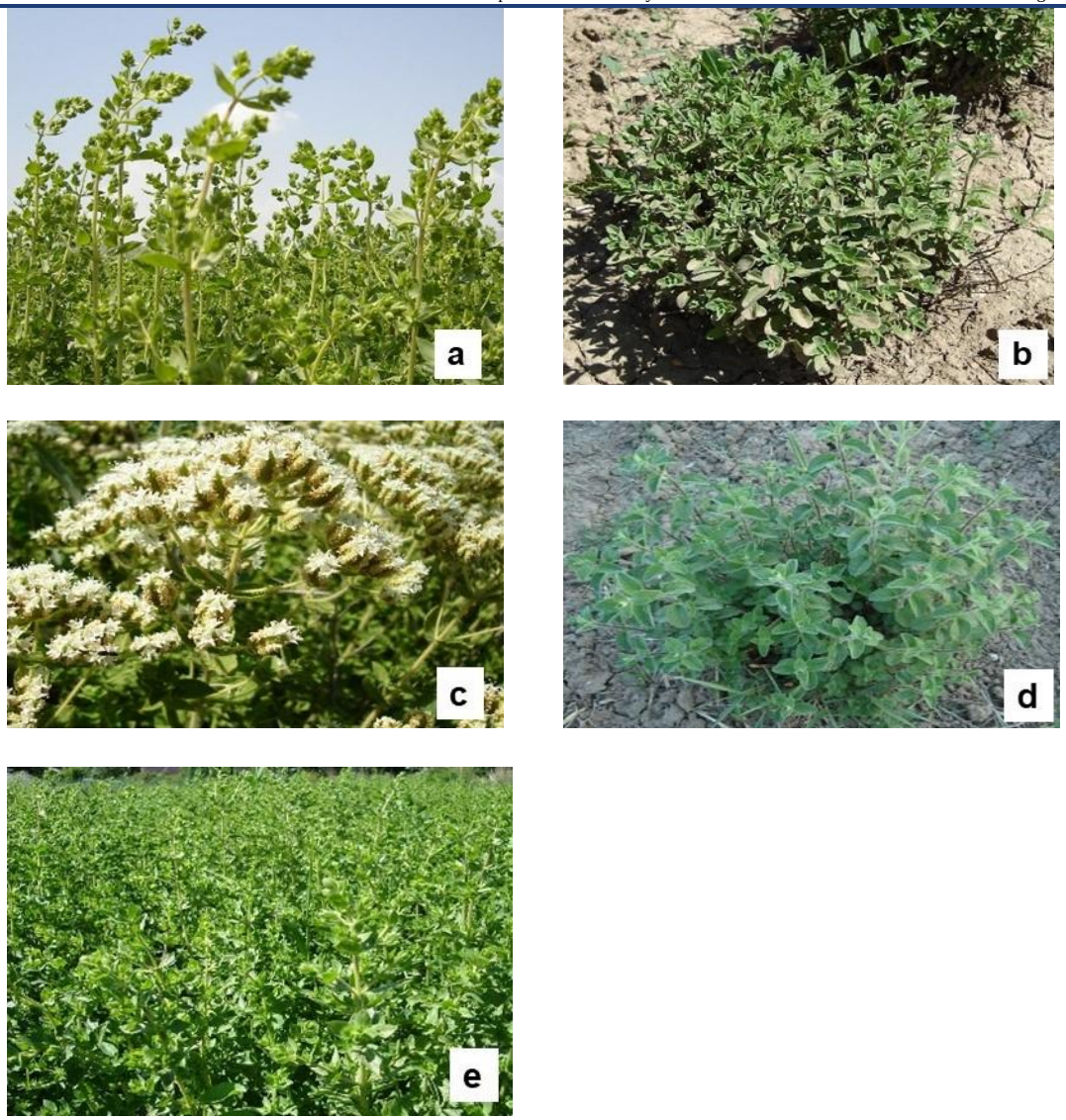

Figure 1. Five species of Origanum. O. vulgare* subsp. *hirtum, a) O. majorana, b) O. onites, c) O. syriacum, d) O. vulgare* subsp. *Vulgare, e) at different stages of growth under field condition

2.1. Soil Physiochemical Characteristics

The climatic parameters of the research region and the physicochemical parameters of the five soils investigated (*Table 1*). The soil pH, was classified as neutral (with a range of 6.5 - 7.5). Due to low EC (less than 2 dS m^{-1}), this soil could not be classified as alkaline. The soil had relatively a loamy texture with high organic carbon (1.15%). These conditions are appropriate for the growing of *Origanum* species. Based on soil nutrient analyses, there was no need of fertilizer application to the soil (K and P concentrations were more than 60 and 15 mg kg^{-1}, respectively). Both organic and inorganic fertilizers are utilized in excess, particularly in Iran, which exceeds the recommended doses (Heydarzadeh et al., 2021a, b). This results in an accumulation of inorganic and organic elements in the soil that eventually degrade with time (Heydarzadeh et al., 2022; Mwadalu et al., 2022; Mirzapour et al., 2022; Amiri et al., 2018).

2.2. Soil Analysis

Five soil samples of studied experimental areas were congregated at random from 0-30 cm deep soil in the experimental fields during autumn using a stainless-steel auger (*Table 2*). The samples were scrupulously mixed and air-dried at a temperature of 25 ^{o}C. This sample was ground and filtered to pass via a two-mm sieve for lab analysis following the methods described by Raudone et al. (2018). Soil electrical conductivity and (EC) pH were evaluated

using 1 soil: 5 water (w/v) ratio with a pH meter (specifically the model of the meter was inolab® pH 7110) and a glass electrode (model 712 conductometer) respectively. Organic matter was measured according to organic matter oxidation using H_2SO_4 and $K_2Cr_2O_7$ then titrating with $FeSO_4$. The soil-available phosphorus (P) is the main indicator for determining soil-plant availability (Mirriam et al., 2022). Briefly, in a 50 ml extraction bottle, 20 ml of $NaHCO_3$ (containing 0.5 mol l^{-1} at a pH of 8.51) and a gram of air-dried soil sample were combined, and the bottles were mechanically shaken for 30 minutes. This was followed by filtration, then P was evaluated by spectrophotometer using the colorimetric technique with ascorbic acid at 820 nm. Available K was assessed using 1 M $C_2H_7NO_2$. The hydrometer method was used to measure particle size distribution (Amiri et al., 2018).

Table 1. Outdoor climatic data of the experiment area*

Month	Year	Average relative humidity	Average monthly temperature	Monthly precipitation
January	2016	63.2	-1.1	59.0
	2017	45.7	-4.4	4.4
February	2016	63.1	4	11.0
	2017	65.4	-4.2	39
March	2016	60.1	7.2	31.3
	2017	54.8	6.3	20.4
April	2016	54.7	12.3	57.9
	2017	56	11.6	59.9
May	2016	57.3	16.6	42.8
	2017	52	17.6	11.9
June	2016	51.4	20.6	28.7
	2017	47.3	22.7	0
July	2016	42.1	24.4	5.5
	2017	40.7	26.3	0.1
August	2016	50.1	24.6	0
	2017	52.4	25.2	0.6
September	2016	62	18.5	0
	2017	63	21.1	0
October	2016	73.1	11.5	16
	2017	69.4	12.6	1.8
November	2016	70.6	4	12.2
	2017	73	6.3	38.4
December	2016	50.7	-2.6	65.6
	2017	48.3	1.7	6.8

*Urmia Meteorology Organization, Urmia, Iran

Table 2. Mean physiochemical characteristics of the studied experimental areas

pH	EC (dS m^{-1})	OC (%)	Olsen-P (mg kg^{-1})	Available -K (mg kg^{-1})	$CaCO_3$ (%)	Sand (%)	Silt (%)	Clay (%)
7.33	0.07	1.15	37.61	165	9.1	45	32	23

2.3. Plant Analysis

For extract preparation, the leaves of *Origanum* species were harvested from the experimental field ensued by chopping and drying at (23 °C) and thereafter after powdering. The extraction solvent was methanol. The extraction process involved adding 2 grams of the soil sample into 25 mL of the solvent which was then mixed at low rpm for 180 minutes. Thereafter, the extract was sieved via a Whatman filter paper sourced from Whatman Ltd., England. A magnetic stirring was used during different steps of the extraction. Sample solutions were concealed with aluminum foil to avoid exposing the sample which was then kept at 4 °C in dark.

2.4. Total Phenolic Content (TPC)

The cumulative phenolic content in leaves was assessed by employing the Folin-Ciocalteau method as documented by Singleton et al. (1999). In this case, 1600 L of distilled water and 10 L of methanolic extracts were combined and treated at 25°C for 5 min with 200 L of Folin-Ciocalteau reagent. For 30 minutes, the mixture was then kept in the dark while being maintained at 25°C after 200 L of sodium carbonate (which was at 7.5%) was added. This was followed by measurement of the TPC quantitates which was determined by reading the sample's absorbance at 760 nm in a DB-20/DB-20S UV/Visible spectrophotometer. TPC was calculated as mg gallic acid g^{-1} dry weight using gallic acid as an external standard.

2.5. Total Flavonoid Content (TFC)

The $AlCl_3$ colorimetric method was used to evaluate the TFC extracts. In brief, 150 L of $NaNO_3$ (5% W/V) was combined with 30 L of the leaves extract and incubated for 5 minutes. After this, 3 mL of aluminum chloride hexahydrate (this was a 10% W/V solution) was added and it was incubated for another 5 minutes. Thereafter, the mixture was dissolved to the desired concentration by addition of the enough mL of 1.0 M NaOH in deionized water. The solution's absorbance was then evaluated using a spectrophotometer calibrated at 510 nm. This was done during a 30-minute incubation at 25°C in the darkness. The external standard for TFC quantification was Quercetin (QE), and TFC was reported as mg QE g^{-1} dry weight (Gayoso et al., 2016).

2.6. Radical Scavenging Activity (RSA)

By employing the colorimetric method reported by Brand-Williams et al. (1995), the RSA of samples was measured. The combination of 2.0 mL of DPPH solution and 15 µL of methanolic extract was equilibrated for 30 min at 20°C in the darkness. The solution's absorbance was evaluated at 517 nm. The DPPH inhibition was computed using the following formula (Elshamy et al., 2018).

$$\text{Inhibition } (\%) = [(\text{Ab control} - \text{Ab sample}) / \text{Ab control}] \times 100 \quad \text{(Eq. 1)}$$

Where $Ab_{control}$ and Ab_{sample} are the respective absorbance of the control and the sample.

2.7. Superoxide Radical Scavenging Activity (SRSA)

For measurement SRSA (Jing et al., 1995), in 9 ml of 5 mM Tris-HCl (buffered at a pH of 8.2), 1 ml of extract was added. Thereafter, 40 µL of 4.5 mM pyrogallol was added. After shaking for 3 min, the solution's absorption spectrum at 420 nm was evaluated using a spectrophotometer. SRSA was then expressed by the oxidation degree of the test group in relation to that of control. Equation 2 was used to determine the percentage of scavenging activity (Amiri et al., 2018; Méabed et al., 2018).

$$\text{SRSA } (\%) = [(\text{Ab0} - \text{Ab1}/\text{Ab}_0)] \times 100 \quad \text{(Eq. 2)}$$

Where Ab_o is the absorbance of the Tris-HCl buffered with pyrogallol whereas Ab_1 denote the absorbance of the extract.

2.8. Nitric Oxide Radical Scavenging Activity (NORSA)

Griess Illovsoy reaction can be used to evaluate nitric oxide radical inhibition (Garrett, 1964). The Griess Illosvoy reagent was altered by substituting naphthyl ethylene diamine dihydrochloride (this was a 0.1% w/v solution) with 1-napthylamine (5%). About 3 mL of a solution containing phosphate buffer saline (0.5 mL), sodium nitroprusside (10 mM, 2 mL), and Artemisia vulgaris extract (25 to 125 mg/mL), was incubated for 150 min at 25°C. After incubation, 1 mL of sulfanilic acid reagent (containing 20% glacial acetic acid) was mixed with 0.5 mL of the solution and left for 5 minutes to complete diazotization to take place. Before being left for 30 min at 25°C, 1 mL of naphthyl ethylene diamine dihydrochloride was added and mixed thoroughly. In diffused light, a pinkish chromophore was observed. Using a spectrophotometer, the absorbance (at 540 nm) of this solution was recorded in comparison to the corresponding blank solutions. Equation 3 was then used to evaluate the radical inhibition of nitric oxide (Tripathy et al., 2017).

$$\text{NORSA } (\%) = [(\text{Ab control} - \text{Ab sample}) / \text{Ab control}] \times 100 \quad \text{(Eq. 3)}$$

Where Ab_{sample} is the absorbance that was recorded in the presence of the samples of the extracts or standards while $Ab_{control}$ was the absorbance from the control.

2.9. Growth Parameters

The growth parameters that were assessed were plant height, herbage dry weight, and plant fresh weight at the full flowering stage. Theses plant samples were oven-dried at 39°C to constant weight which was achieved after 48 hours.

2.10. Essential Oil (EO) and Essential Oil Yield (EOY)

Extraction of EO was done according to the method of distillation with water and using a Cloninger device. In order to collect the essential oil, after opening the valve and draining the water, the essential oil was collected in a small bottle that was weighed with a digital scale of 0.0001. Then these bottles were weighed and the weight of essential oil per 100 grams of dry leaves was calculated (Adams et al., 2007). EOY was calculated using Equation 4.

$$\text{EOY} = \text{EO} \times \text{herbage dry weight} \quad \text{(Eq. 4)}$$

2.11. Gas Chromatographic-mass Spectrometric Analysis of Essential Oil

Using a Clevenger-type device, dried aerial portions of the plants were hydro-distilled for 3 hours in 500 mL water. Gas Chromatography (GC) analysis was performed to distinguish different compounds in essential oil. The Hewlett Packard 6890 N GC with FID detector and an HP 5MS 30 m by 0.25 mm by 0.25 μm film thickness capillary column was used for all GC examination. The temperature of the column was controlled to increase from 50°C to 150°C at a frequency of 3°C min^{-1}. Temperatures rise were set at 220 °C and 290 °C for calibration of the injector and detector, respectively. Helium was equally used as a carrier gas at a constant flow rate of 1 mL min^{-1}. Analyses of the gas chromatography-mass spectrometry (GC/MS) were executed using mass selective detector (a Hewlett Packard 5973)-6890 GC/MS system which was operating in the electron ionization structure with 70 eV ionization energy. This was equipped with film thickness capillary column with dimensions of HP 5MS 30 m by 0.25 mm by 0.25 μm. Here the carrier gas used was He (1 mL min^{-1}). The initial temperature of the column was set at 50 °C, with a gradual increase in heat to 150 °C at a frequency of 3 °C min^{-1}. This temperature was maintained for 10 min after which it was escalated to 250 °C min^{-1}. Thereafter, automatic injection of diluted of 1.0 μL of samples (1/100 in acetone, v/v) was done in the spitless mode. The chemical substances identified in this research were identified by comparing their retention durations and mass spectra to those derived from the Flavor 2. L, NIST98.L, and Wiley7n.1 spectral and literature records (Pezzani et al., 2017; Sefeer et al., 2018). FID chromatograms were used to evaluate the relative percentages of the separated substances.

2.12. Statistical Analysis

For this study, Info Stat software, version 2010 was used for data analysis. The standard deviations and means were determined. To discover significant differences between means, ANOVA (analysis of variance) and the LSD (Fisher's multiple range) test were used. Pearson's test was used to do the correlation analysis.

3. Results and Discussion

The various species of *Origanum* were significant for the total phenol, total flavonoid, DPPH radical scavenging capacity, superoxide radicals scavenging capacity, nitric oxide radicals scavenging and chain-breaking capacity, plant height, fresh weight, dry weights, essential oil, and essential oil yield (*Table 3*).

3.1. Total Phenolic Compounds (TPC)

The TPC forms a key diverse group of plant secondary metabolites which are linked to numerous ecophysiological conditions. The TPC of *Origanum* species, determined by the Folin-Ciocalteau colorimetric method, is shown in *Table 4*, which ranged from 36.21 to 51.12 mg GAE g^{-1} dry weight. For TPC, there were considerable variances among the investigated species. The utmost and the lowermost TPC levels were observed in *O. vulgare* subsp. *hirtum* and *O. syriacum* species, respectively. Comparing the results of this study with others noted that TPC in Iranian *Origanum* species was 2 times higher compared to the same species collected under Turkish conditions (Morshedloo et al., 2018; Raudone et al., 2018). Moghrovyan et al. (2019) demonstrated that climate conditions affected the antioxidant activities and TPC contents. Changes in phenolic chemicals in various species may be caused by genetic background and growth circumstances (Elshamy et al., 2018). Environmental variables have a paramount influence on phenolic content (such as rainfall, soil composition, temperature, and UV radiation) (Gayoso et al., 2018). Climate change, predators, disease invasion, nutritional restriction, and excessive

irradiation can increase not only the reactive oxygen species (ROS) but also the free radicals (Ngugi et al., 2021). This results in increased antioxidants in plants such as phenolic compounds (Marrelli et al., 2018; Koleva et al., 2018). It is widely presumed that an enzyme which is found in various plant species called phenylalanine ammonia-lyase (PAL) functions as a prominent indicator of environmental stress factors in plants.

Table 3. The results of the analysis of variance for various Origanum species

Sources of variations	df	Total phenol	Total flavonoid	DPPH radical scavenging	Superoxide radicals scavenging	Nitric oxide radicals scavenging	Chain breaking	Plant height	Fresh weight	Dry weights	Essential oil	Essential oil yield per plant	essential oil yield per hectare
Block	2	1.25	0.40	0.42	34.82	6.41	13.82	5.56	5415	695.54	0.08	0.003	17.81
Origanum species	4	87.72**	4.49**	108.82**	330.14**	18.75*	416.26**	1.839,53**	307.655,06**	28.102,64**	4.07**	0.44**	1.999,39**
Error	8	13.39	0.27	13.71	11.67	3.94	10.49	4.12	1.773,16	285.89	0.23	0.02	94.05
C.V (%)		8.89	10.92	7.42	10.16	11.32	11.43	3.19	9.55	12.23	14.49	13.42	13.47

ns, * and ** show non-significance and significance at the $p < 0.05$ and $p < 0.01$ level, respectively.

Table 4. Total phenolic compounds of various Origanum species

Compounds	Species				
	O. majorana	***O. onites***	***O. syracum***	***O. vulgare subsp. vulgare***	***O. vulgare subsp. hirtum***
TPC (%)	41.12±0.56	43.22 ±0.40	36.21±0.61	41.59±0.88	51.12 ±0.32
TFC (%)	4.23 ±0.25	4.43 ±0.33	3.93 ±0.38	4.30 ±0.21	6.93 ±0.44

3.2. Total Flavonoid Contents (TFC)

Flavonoids are plants' bioactive chemical components that can be identified in almost every part of the plant. They are fundamental for floral coloration and fragrance, as well as for protecting plants from UV degradation. Consequently, UV radiation significantly increases flavonoid synthesis (Moreno et al., 2018). There were considerable differences among the investigated species for TFC. In this case, TFC of the five *Origanum* species ranged from 3.93 (in *O. syriacum*) to 6.93 (in *O. vulgare* subsp. *hirtum*) mg QE g^{-1} dry weight (*Table 4*). Differences among the *Origanum* species in TFC may be attributed to variances in the species' genetic background. The results of the current study are in line with previous findings (Amiri et al., 2018; Méabed et al., 2018). Bonea and Urecheam, (2018) determined that the flavonoid content of three *Origanum* species was around 2.14-4.29 mg QE g^{-1} dry weight. These differences are presumed due to the plant genotype and their interaction with the local climatic conditions.

3.3. Total Antioxidant Activity

There are several mechanisms used for measuring antioxidant activities. In the current project, the antioxidant properties of the *Origanum* species were assessed using the DPPH method. The scavenging activity of DPPH radicals in leaf extracts of these species ranged from 39.47 to 53.79% (*Table 5*). In the DPPH assay, the uppermost antioxidant activity was noted in *O. vulgare* subsp. *hirtum* whereas the least activity by DPPH assay was obtained in *O. syriacum.* Analogous results were found during the measurement of nitric oxide and superoxide antioxidant activities. The *O. vulgare* subsp. *hirtum* species showed the highest nitric oxide and superoxide activity among all species at 21.60% and 50.04% (*Table 5*).

Table 5. Total antioxidant activity of different Origanum species

Species	*O. majorana*	*O. onites*	*O. syriacum*	*O. vulgare* subsp. *vulgare*	*O. vulgare* subsp. *hirtum*
DPPH (%)	50.56 ±1.20	54.29 ±1.09	39.47 ±1.35	51.14 ±0.99	53.79 ±1.17
Nitric oxide (%)	15.13 ±0.05	16.62 ±0.08	16.26 ±0.04	18.05 ±0.07	21.60 ±0.04
Superoxide (%)	30.36 ±1.32	35.18 ±1.56	21.29 ±1.11	31.27 ±1.73	50.04 ±2.00

3.4. Plant height

The highest plant height (86.4 cm) was observed in *O. vulgare* subsp. *hirtum.* Also, *O. vulgare* subsp. *Hirtum* and *O. syriacum* species had statistically similar effects on plant height. The lowest plant height (32.6 cm) was obtained in *O. majorana* (*Figure 2*).

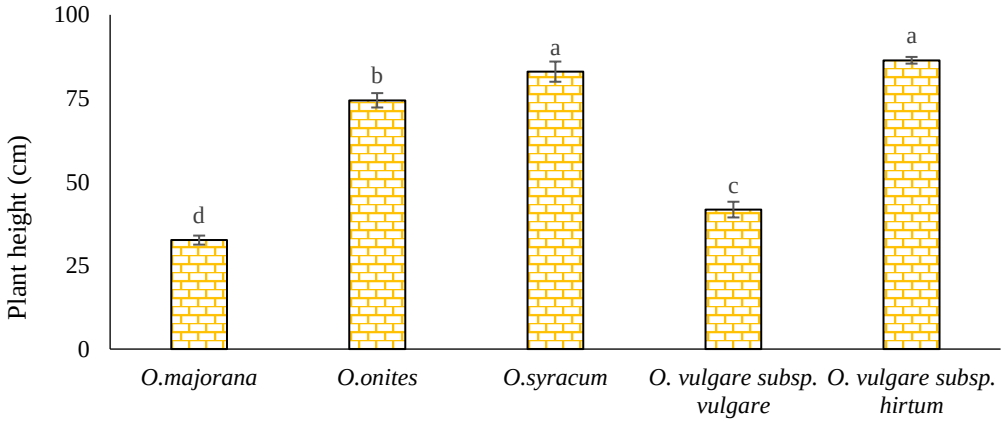

Figure 2. Plant height of 5 Origanum species used in the current study

3.5. Plant fresh weight

The mean comparison for the *Origanum* species revealed that the highest plant fresh weight of 826 g was obtained from *O. onites*, which did not differ significantly from *O. vulgare* subsp. *hirtum* species. The lowest one (92 g) was obtained from the *O. majorana* species (*Figure 3*).

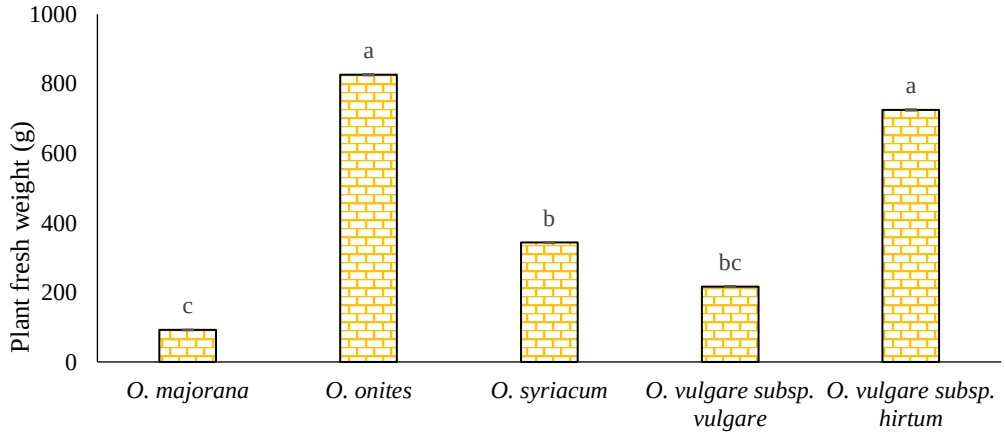

Figure 3. Plant fresh weight of 5 Origanum species used in the current study

3.6. Dry weight

The highest dry weight (250 g) was observed in *O. onites*, Also, *O. onites* and *O. vulgare* subsp. *hirtum* species had statistically similar effects on dry weights. The lowest dry weight (50 g) was obtained in *O. majorana* (*Figure 4*).

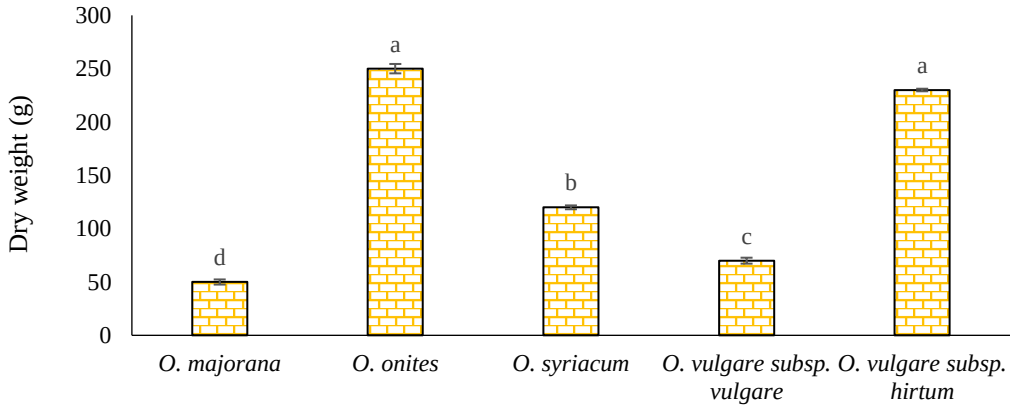

Figure 4. Dry weights of 5 Origanum species used in the current study

Investigated subspecies differed in morphological traits (*Figure 2, 3, 4*). Obtained results indicate on significant differences between examined *Origanum* subspecies and correspond well with the literature data (Węglarz et al., 2020). However, it should be underlined that each subspecies is very variable itself and its morphological features strongly depend on the population/accession origin (Kosakowska et al., 2019). Observed phenotypical plasticity may be related to allogamous way of this plant's reproduction as well as its heterozygous character. Traits such as type of growth habit, lignification degree as well as branching, and foliar density can be important from the practical viewpoint since they affect the yield of herbs and enable their mechanical harvest. In the present study, the plant height, fresh and dry weight of *Origanum* herb species had a significant difference (*Figure 2, 3, 4*). Such results may be related to the temperature requirements of *Origanum* herb species resulting from its origin (Węglarz et al., 2020).

3.7. Essential Oil Percentage

The highest essential oil percentage was 5.26% (*Figure 5*), which was observed in *O. vulgare* subsp. *hirtum* species. The lowest one (2.06%) was obtained from *O. syriacum* species, which did not differ significantly from *O. majorana, O. onites, and O. vulgare* subsp. *vulgare* species (*Figure 5*).

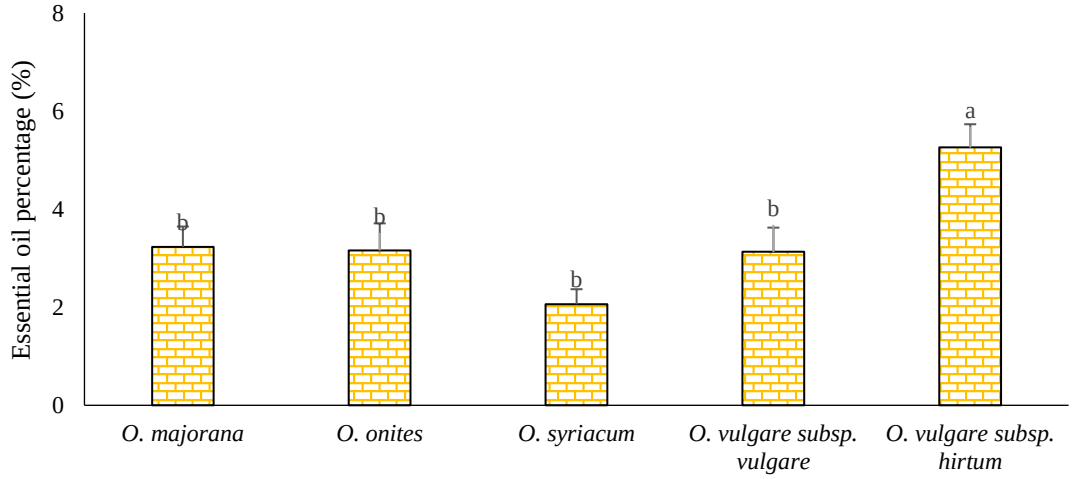

Figure 5. Essential oil percentage of 5 Origanum species used in the current study

3.8. Essential Oil Yield per Plant

The utmost EOY per plant was 1.71 g (*Figure 6*), which was obtained in *O. vulgare* subsp. *Hirtum* species. The lowest one (0.66 g) was obtained from an *O. syriacum* species. Also, *O. majorana, O. vulgare* subsp. *vulgare,* and *O. onites* species had statistically similar essential oil yields as that of *O. syriacum* species.

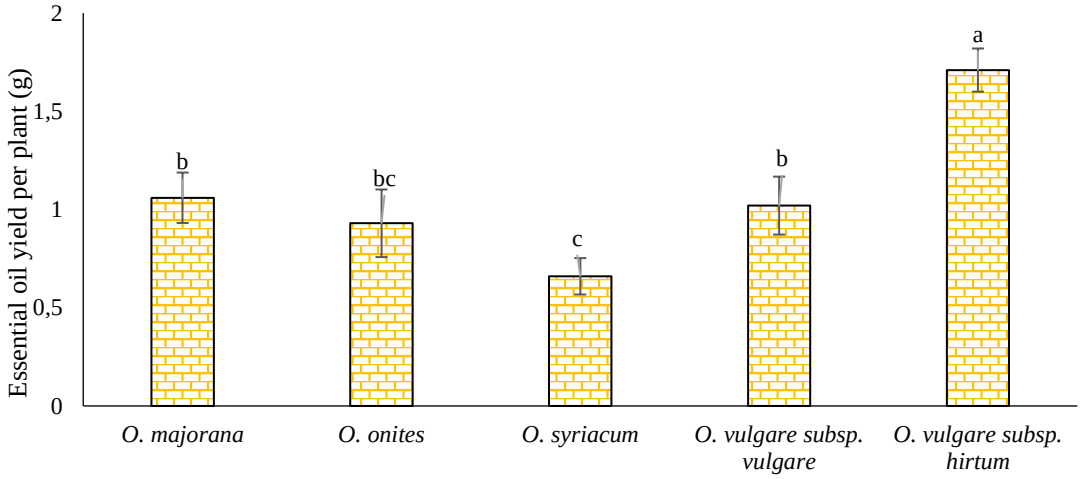

Figure 6. Essential oil yield per plant of 5 Origanum species used in the current study

3.9. Essential Oil Yield per ha

Differences in the essential oil yield were observed in *Origanum* species in the present study. The lowest essential oil yield (44 kg ha^{-1}) was recorded in *O. syriacum* whereas the highest yield (114 kg ha^{-1}) was observed in *O. vulgare* subsp. *hirtum* (*Figure 7*).

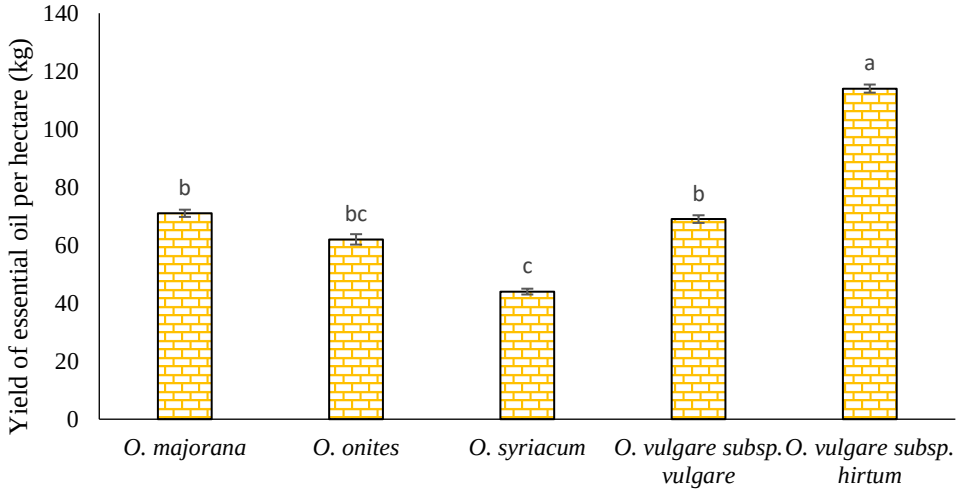

Figure 7. The yield of essential oil per hectare in different Origanum species

In *Origanum* subspecies (as well as in other *Lamiaceae*), glandular trichomes are multicellular epidermal glands responsible for storage of essential oil. Two different types of these glands were recognized on the epidermis of *Origanum* species: peltate and capitate glands (Węglarz et al., 2020). The glandular trichomes are built of one basal cell, one stalk cell and a multi-cellular head, where essential oil is synthesized before being transferred to subcuticular area (Kosakowska et al., 2019). Svidenko et al. (2018) claim that the location of glandular trichomes have valuable taxonomical significance at the species level. In the present work, it is most likely due to the variable formation of glandular trichomes among *Origanum* species, leading to differences in the amount of essential oil (*Figure 5, 6, 7*). This pattern corresponds with studies undertaken earlier by Shafiee-Hajiabad et al., (2014). However, the formation of glandular trichomes is variable and can be controlled by both genetic and environmental factors (Kosakowska et al., 2019), It has led to a difference in the amount of essential oil. It is worth noting that the relationship between the number of glandular trichomes and essential oil content has been found (*Tables 4* and *5*), what refers to results shown by Shafiee-Hajiabad et al. (2014).

3.10. Principal Component Analysis (PCA)

The PCA for the assessed variables on essential oil, antioxidant compounds and quantitative traits for 5 *Origanum* species *O. majorana*, *O. vulgare* subsp. *vulgare*, *O. syriacum*, *O. onites*, and *O. vulgare* subsp. *hirtum*) resulted in 67 and 21% variation being accounted for by the first (PC1) and second (PC2) principal components, respectively (*Figure 8*). The variability in PC1 was mainly due to two groups of variables. The first one consisted of the quantitative traits measured by *O. onites* species form a group characterized by plant height, plant fresh weight, and plant dry weight. The second group consists of *O. vulgare* subsp. *hirtum* species, distinguished by higher amounts of phenolic compounds, flavonoid contents, DPPH scavenging activity radical, nitric oxide, superoxide, essential oil based on percentage, per hectare, and per plant. The second principal component (PC2) was weighted by percentage biomass, and the projection of the variances based on the PCA revealed a significant separation of this variable from the rest along the first axis.

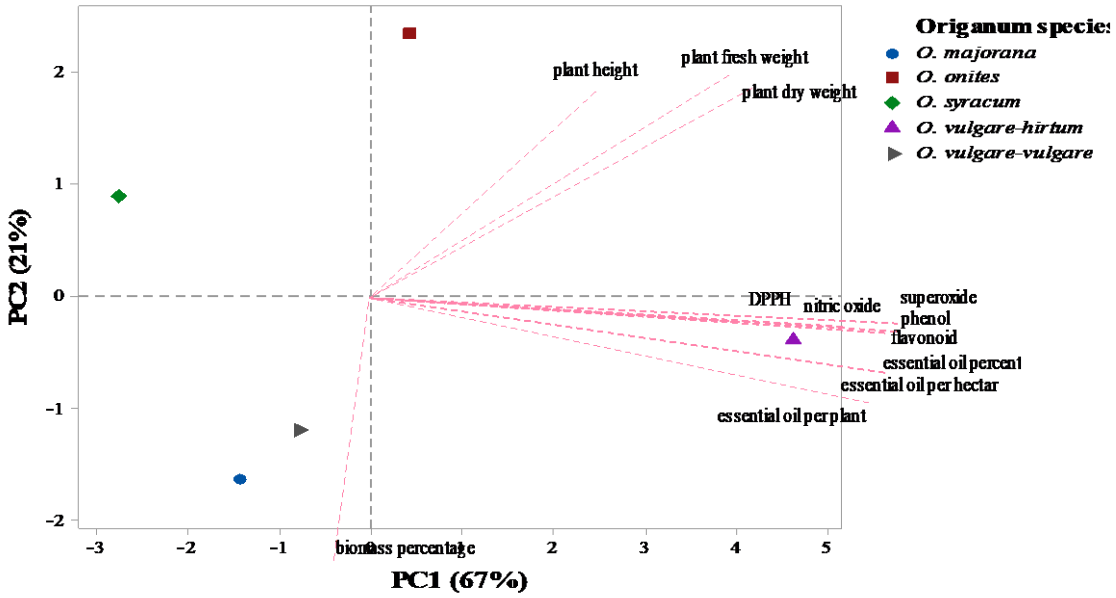

Figure 8. Principal component analysis of various origanum species

3.11. Cluster analysis

The results acquired from the cluster analysis indicated the presence of high inter-production variability in the antioxidant compounds of various *Origanum* species. From the 5 production samples presented to a multivariate evaluation, well-defined groups of antioxidant compounds were distinguished by using cluster analysis (*Figure 9*). According to the information, two subclusters were obtained: The first subset included three *Origanum* species which were *O. vulgare* subsp. *vulgare*, *O. majorana*, and *O. syriacum*, and the second subset had two *Origanum* species: *O. onites*, and *O. vulgare* subsp. *hirtum*.

3.12. Essential Oil Analysis

Various essential oil components were analyzed based on the GC-MS analysis. thymol (6.90-59.89%), carvacrol (0.83-48.91%), γ-terpinene (6.55-18.20%), p-cymene (0.50-20.94%) and α-terpinene (2.71-4.28%) were the most prominent constituents in *Origanum* plants (*Table 6*). The uppermost level of Thymol was detected in *O.majorana* and the lowest amount of it was obtained from *O.vulgare-vulgare*. The peak level of carvacrol (48.91%) was noted in *O. vulgare* subsp. *hirtum* and the lowest amount of it (0.8%) was obtained from *O. syriacum.*

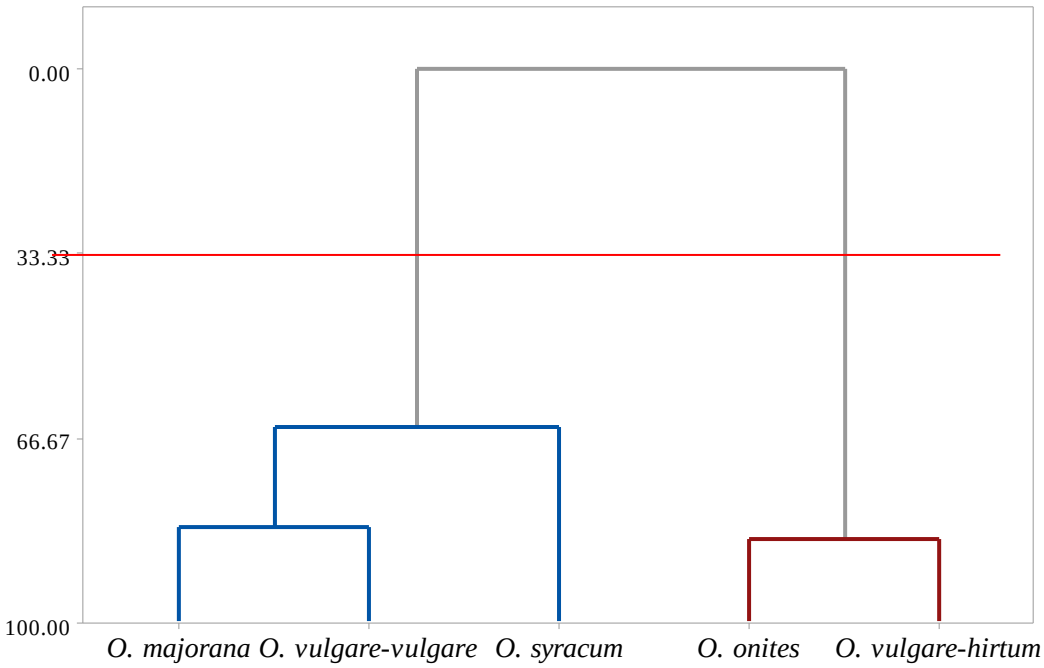

Figure 9. Cluster analysis of 5 Origanum species

As per this study, the antioxidant properties and essential oil content of *Origanum* varied among species (*Table 6*). The density of oil glands involving plant tissues, alteration in the growing environment of plants as the plant matures, and changing allocation of carbohydrates to plant development and growth rather than essential oil synthesis could all cause alterations in essential oil composition. For instance, medicinal plants regulate essential oil production and the attraction of pollinators while repelling herbivores and pests (Moghrovyan et al., 2019; Pezzani et al., 2017). In our study, thymol and carvacrol were the most common component of the essential oil in the *O. majorana* and *O.vulgare* subsp. *hirtum*, respectively. Other *oregano* species including *O. vulgare* subsp.*hirtum* (Moreno et al., 2018), European *O. vulgare* (Duletić-Laušević et al., 2018), *O. vulgare* subsp. *glandulosum* (Gayoso et al., 2018), O. *vulgare* (Pezzani et al., 2017) have similarly showed comparatively high levels of carvacrol. The current study confirms that the highest level of carvacrol (48.91%) was obtained from *O. vulgare* subsp. *hirtum*. Ramakrishna et al. (2019) found carvacrol as a dominant component in the essential oil of *O. vulgare* subsp. *hirtum*. These findings show that the gene for carvacrol generation is preserved. The monoterpenes, g-terpinene, p-cymene, and hydrocarbons operate as progenitors for thymol, and carvacrol thus their rates reduce with an increase in the levels of carvacrol (Sefeer et al., 2018). It is known that many various factors can affect the content and composition of essential oils in aromatic plants, where the most seem to be: genetic, physiological and environmental including temperature, intensity of solar and radiation humidity (Węglarz et al., 2020).

4. Conclusions

The present study investigated the phytochemical properties of five species (*Origanum* species including *O.majorana*, *O. syriacum*, *O. onites*, *O. vulgare* subsp. *vulgare* and *O. vulgare* subsp. *hirtum*) of marjoram (*Origanum majorana* L.) an important ethnomedicinal plant. Total phenolic compounds, total flavonoid content, total antioxidant activity, and essential oil compounds were determined. The qualitative analysis showed the highest (240 g) dry weight of *O. vulgare* subsp. *hirtum* and *O. onites* species in comparison with others. The quantitative analysis revealed the higher content of total phenol (51.12%), flavonoid (6.93%), DPPH (54.29%), superoxide (50.04%) and radical scavenging activity in *O. vulgare* subsp. *hirtum* species, but the *O. onites* showed higher (21.60%) nitric oxide radical scavenging activity. The essential oil analysis revealed that the thymol (6.90-59.89%), carvacrol (0.83-48.91%), γ-terpinene (6.55-18.20%), p-cymene (0.50-20.94%) and α-terpinene (2.71-

4.28%) were the most prominent constituents in *Origanum* plants. Cluster analysis showed two main categories and high similarity between *O. vulgare* subsp. *hirtum* and *O. onites.* In conclusion, the *O. vulgare* subsp. *hirtum* was the best species in terms of phytochemical constituents.

Table 6. Different compounds of essential oil in various Origanum species.

Component	Peak area ((%)				
	O. majorana	***O. onites***	***O. syriacum***	***O. vulgare* subsp. *vulgare***	***O. vulgare* subsp. *hirtum***
α-phellandrene	1.29	1.41	1.17	0.27	1.35
α-pinene	0.52	0.74	0.68	0.45	0.66
sabinene	-	-	-	3.47	-
1-octen-3-ol	0.47	0.44	-	-	0.24
myrcene	1.77	1.57	1.50	1.22	1.47
3-octanol	-	0.39	-	-	-
δ-3-carene	0.37	0.35	0.50	-	0.30
α-terpinene	3.82	4.28	2.71	3.79	2.78
p-cymene	11.99	20.94	15.52	0.50	10.69
cyclohexene	0.65	0.63	-	1.28	-
beta-Pinene	-	-	-	0.29	0.46
eucalyptol	-	-	-	0.26	-
γ-terpinene	11.65	15.55	18.20	6.55	8.18
trans-sabinene hydrate	-	0.70	-	4.32	0.26
terpinolene	-	-	-	1.49	-
cis-sabinene hydrate	-	-	-	36.38	-
isoborneol	0.61	-	0.56	0.44	0.40
terpinen-4-ol	1.04	0.64	0.43	16.12	0.77
alpha-Terpineol	-	-	-	5.40	-
carvacrol methyl ether	-	-	3.63	-	2.05
tricyclene	-	-	-	2.27	-
thymol	59.89	43.24	51.55	6.90	19.26
Carvacrol	3.55	8.38	0.83	1.76	48.91
β-caryophyllene	1.88	0.76	1.58	1.35	1.77
b-bisabolene	0.51	-	1.14	-	0.43

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Ethical Statement

There is no need to obtain permission from the ethics committee for this study.

Conflicts of Interest

We declare that there is no conflict of interest between us as the article authors.

Authorship Contribution Statement

Authors contributed equally.

References

Adams, R. P. (2007). Identification of Essential oil Components by gas Chromatography/Quadrupole Mass Spectrometry, 4th ed. Allured Publ. Corp, Carol Stream.

Aktepe, B. P., Mertoğlu, K., Evrenosoğlu, Y. and Aysan, Y. (2019). Determination of antibacterial effect of different plant essential oils against *Erwinia amylovora*. *Journal of Tekirdag Agricultural Faculty*, 16: 34-41. https://doi.org/10.33462/jotaf.516848

Amiri, N., Yadegari, M. and Hamedi, B. (2018). Essential oil composition of *Cirsium arvense* L. produced in different climate and soil properties. *Records of Natural Products*, 12: 251.-262. https://doi.org/10.25135/rnp.27.17.06.043

Bhardwaj, K. and Dubey, W. (2019). Quantitative analysis of primary and secondary metabolites of ethanol seed extract of *Origanum majorana* (Marjoram). *Journal of Pharmacogn Phytochem*, 8:1251-1255.

Bonea, D. and Urechean, V. (2018). Effect of sweet marjoram (*Origanum majorana* L.) cogermination and aqueous extracts on maize (*Zea mays* L.). *Agriculture for Life, Life for Agriculture*, 1: 11-16. https://doi.org/10.2478/alife-2018-0002

Brand-Williams, W., Cuvelier, M.E. and Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology*, 28:25-30.

Duletić-Laušević, S., Aradski, A.A., Kolarević, S., Vuković-Gačić, B., Oalđe, M., Živković, J., Šavikin, K.and Marin, P.D. (2018). Antineurodegenerative, antioxidant and antibacterial activities and phenolic components of *Origanum majorana* L. (Lamiaceae) extracts. *Journal of Applied Botany and Food Quality*, 91: 126-134. https://doi.org/10.5073/JABFQ.2018.091.018

Elshamy, A.I., Mohamed, T.A., Marzouk, M.M., Hussien, T.A., Umeyama, A., Hegazy, M.E.F. and Efferth, T. (2018). Phytochemical constituents and chemosystematic significance of *Pulicaria jaubertii* E. Gamal-Eldin (Asteraceae). *Phytochemistry Letters*, 24: 105-109. https://doi.org/10.1016/j.phytol.2018.01.021

Faridvand, S., Rezaei-Chiyaneh, E., Battaglia, M., Gitari, H., Raza, M.A. and Siddique, K.H.M. (2021). Application of bio and chemical fertilizers improves yield, and essential oil quantity and quality of Moldavian balm (*Dracocephalum moldavica* L.) intercropped with mung bean (*Vigna radiata* L.). *Food and Energy Security*, p. e319. https://doi.org/10.1002/fes3.319

Garrett D. (1964). The quantitative Analysis of Drugs. Chapman and Hall, Japan, 3: 456-458.

Gayoso, L., Claerbout, A.S., Calvo, M.I., Cavero, R.Y., Astiasarán, I. and Ansorena, D. (2016). Bioaccessibility of rutin, caffeic acid and rosmarinic acid: Influence of the in vitro gastrointestinal digestion models. *Journal of Functional Foods*, 26: 428-438. https://doi.org/10.1016/j.jff.2016.08.003

Gayoso, L., Roxo, M., Cavero, R.Y., Calvo, M.I., Ansorena, D., Astiasarán, I. and Wink, M. (2018). Bioaccessibility and biological activity of *Melissa officinalis*, Lavandula latifolia and *Origanum vulgare* extracts: Influence of an in vitro gastrointestinal digestion. *Journal of Functional Foods*, 44: 146-154. https://doi.org/10.1016/j.jff.2018.03.003

Hassan, M.J., Raza, M.A., Rehman, S.U., Ansar, M., Gitari, H., Khan, I., Wajid, M., Ahmed, M., Shah, G.A., Peng, Y. and Li, Z. (2020). Effect of cadmium toxicity on growth, oxidative damage, antioxidant defense system and cadmium accumulation in two sorghum cultivars. *Plants*, 9(11): 1575. https://doi.org/10.3390/plants9111575

Heydarzadeh, S., Gitari, H., Rahimi, A., Khiavi, H.K., Maitra, S. And Hosseinpour, A. (2021a). Yield and quality traits of field-grown sugar beet (*Beta vulgaris* L.) in response to foliar application of micronutrients and different levels of manure. *International Journal of Bioresource Science*, 8(2): 103–112.

Heydarzadeh, S., Hosseinpour, A., Gitari, H., Maitra, S. and Rahimi, A. (2021b). Improving quantitative and qualitative properties of cotton through the use of organic and chemical fertilizers under water deficit stress conditions. *International Journal of Bioresource Science*. 8(2): 69–80.organic

Heydarzadeh, S., Jalilian, J., Pirzad, A., Jamei, R. and Petrussa, E. (2022). Fodder value and physiological aspects of rainfed smooth vetch affected by biofertilizers and supplementary irrigation in an agri-silviculture system. *Agroforestry Systems*, 96: 1-12.

Jing, T.Y. and Zhao, X.Y. (1995). The improved pyrogallol method by using terminating agent for superoxide dismutase measurement. *Progress in Biochemistry and Biophysics*, 22: 84-86.

Kaplan, M., Mücahit Yilmaz, M., Uslu, R., Köprü, S., Gözelle, H. and Muhderem, G. (2019). An optimization study for the production of *Origanum onites* tincture by response surface methodology: effect of liquid/solid ratio, ethanol concentration and storage period. *Journal of Tekirdag Agricultural Faculty*, 16: 11-22.

Koleva, V., Dragoeva, A., Stoyanova, Z., Yordanova, Z., Ali, S., Uzunov, N.M., Melendez-Alafort, L., Rosato, A. and Enchev, D.D. (2018). In vitro cytotoxicity of allelopathic plants L. ssp. L. and subsp. *Acta Scientiarum Naturalium*, 5: 64-69.

Kosakowska, O., Węglarz, Z. and Bączek, K. (2019). Yield and quality of 'Greek oregano' (*Origanum vulgare* L. subsp. *hirtum*) herb from organic production system in temperate climate. *Industrial Crops and Products*, 141(10):111782 https://doi.org/10.1016/j.indcrop.2019.111782

Marrelli, M., Statti, G.A. and Conforti, F. (2018). *Origanum* spp.: an update of their chemical and biological profiles. *Phytochemistry Reviews*, 17:873-888.

Méabed, E.M., El-Sayed, N.M., Abou-Sreea, A.I. and Roby, M.H. (2018). Chemical analysis of aqueous extracts of *Origanum majorana* and *Foeniculum vulgare* and their efficacy on *Blastocystis* spp. cysts. *Phytomedicine*, 43: 158-163.

Mirriam, A., Mugwe, J., Raza, M.F., Seleiman, M.A., Maitra, S. and Gitari, H.I. (2022). Aggrandizing soybean yield, phosphorus use efficiency and economic returns under phosphatic fertilizer application and inoculation with *Bradyrhizobium*. *Journal of Soil Science and Plant Nutrition*, 22: 5086- 5098. https://doi.org/10.1007/s42729-022-00985-8

Mirzapour, M., Valizadeh, N., Heydarzadeh, S., Rahimi, A. and Gitari, H. (2022). Factors Affecting the Production of Secondary Metabolites in Medicinal Plants. Pg 3-32. In: Ozyazıcı G (Ed). New Development on Medicinal and Aromatic Plants-II, Iksad publishing house, Turkey.

Moghrovyan, A., Sahakyan, N., Babayan, A., Chichoyan, N., Petrosyan, M. and Trchounian, A. (2019). Essential oil and ethanol extract of oregano (*Origanum vulgare* L.) from armenian flora as a natural source of terpenes, flavonoids and other phytochemicals with antiradical, antioxidant, metal chelating, tyrosinase inhibitory and antibacterial activity. *Current Pharmaceutical Design*, 25: 1809-1816.

Moreno, Y. and Arteaga-Miñano, H. (2018). Natural conservation of guinea pig (*Cavia porcellus*) meat vacuum packed: Oregano essential oil effect on the physicochemical, microbiological and sensory characteristics. *Scientia Agropecuaria*, 9: 467-476.

Morshedloo, M. R., Salami, S.A., Nazeri, V., Maggi, F. and Craker, L. (2018). Essential oil profile of oregano (*Origanum vulgare* L.) populations grown under similar soil and climate conditions. *Industrial Crops and Products*, 119: 183-190.

Mwadalu, R., Mochoge, B., Mwangi M., Maitra S. and Gitari, H. (2022). Response of Gadam sorghum (*Sorghum bicolor*) to farmyard manure and inorganic fertilizer application. *International Journal of Agriculture, Environment, and Biotechnology*, 15: 51–60. https://doi.org/10.30954/0974-1712.01.2022.6

Ngugi, M. M., Gitari, H. I., Muui C. and Gweyi-Onyango, J. P. (2021). Cadmium mobility, uptake and accumulation in spinach, kale and amaranths vegetables as influenced by Silicon fertilization. *Bioremediation Journal*, 26(2): 113–127. https://doi.org/10.1080/10889868.2021.1924111

Oke-Altuntas, F., Demirci, M.A., Demirtas, I., Yaglioglu, A. S. and Behcet, L. (2018). Phytochemical screening, antiproliferative and antioxidant properties of various extracts from endemic origanum acutidens. *Combinatorial Chemistry & High Throughput*, 21: 281-291.

Pandey, A., Belwal, T., Tamta, S., Bhatt, I. and Rawal, R. (2019). Phenolic compounds, antioxidant capacity and antimutagenic activity in different growth stages of in vitro raised plants of *Origanum vulgare* L. *Molecular Biology Reports*, 46:2231-2241. https://doi.org/10.1007/s11033-019-04678-x

Pezzani, R., Vitalini, S. and Iriti, M. (2017). Bioactivities of *Origanum vulgare* L.: an update. *Phytochemistry Reviews*, 16: 1253-1268.

Rahimi, A., Mohammadi, M.M., Moghadam, S.S., Heydarzadeh, S. and Gitari, F. (2022). H. Effects of stress modifier biostimulants on vegetative growth, nutrients and antioxidants contents of garden thyme (*Thymus vulgaris* L.) under water stress. *Journal of Plant Growth Regulation*, 41: 2059–2072. https://doi.org/10.1007/s00344-022-10604-6

Ramakrishna, R., Sarkar, D. and Shetty, K. (2019). Metabolic stimulation of phenolic biosynthesis and antioxidant enzyme response in dark germinated barley (*Hordeum vulgare* L.) sprouts using bioprocessed elicitors. *Food Science and Biotechnology*, 28: 1093-1106.

Raudone, L., Motiekaityte, V., Vainoriene, R., Zymone, K., Marksa, M. and Janulis, V. (2018). Phytochemical profiles of alpine plant *Horminum Pyrenaicum* L. during phenological growth stages. *Chemistry & Biodiversity*, 15: e1800190. https://doi.org/10.1002/cbdv.201800190

Salvo, A., La Torre, G.L., Rotondo, A., Cicero, N., Gargano, R., Mangano, V., Casale, K.E. and Dugo, G. (2018). Multiple analytical approaches for the organic and inorganic characterization of *Origanum vulgare* L. samples. *Natural Product Research*, 33: 2815-2822. https://doi.org/10.1080/14786419.2018.1503269

Sefeer, K. and Elumalai, K. (2018). Larvicidal activity of plant oil, *Origanum majorana* L. against the selected mosquito larvae and GC-MS analysis of its phytochemical compounds. *Annals of the Entomological Society of America*, 36: 01-05.

Shafiee-Hajiabad, M., Hardt, M. and Honermeier, B. (2014). Comparative investigation about the trichome morphology of Common oregano (*Origanum vulgare* L. subsp. *vulgare*) and Greek oregano (*Origanum vulgare* L. subsp. *hirtum*). *Journal of Applied Research on Medicinal and Aromatic Plants*, 1(2): 50-58. https://doi.org/10.1016/j.jarmap.2014.04.001

Singleton, V.L., Orthofer, R. and Lamuela-Raventós, R.M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods in Enzymology*, 299: 152-178.

Spyridopoulou, K., Fitsiou, E., Bouloukosta, E., Tiptiri-Kourpeti, A., Vamvakias, M., Oreopoulou, A., Papavassilopoulou, E., Pappa, A. and Chlichlia, K. (2019). Extraction, chemical composition, and anticancer potential of *Origanum onites* L. essential oil. *Molecules*, 24(14):2612. https://doi.org/10.3390/molecules24142612

Svidenko, L., Grygorieva, O., Vergun, O., Hudz, N., Horˇcinová, E., Sedláˇcková, V., Šimková, J. and Brindza, J. (2018). Characteristic of leaf peltate glandular trichomes and their variability of some Lamiaceae family species. *Agrobiodiversity*, 10: 124–131. https://doi.org/10.15414/agrobiodiversity.2018.2585-8246.124-132

Tripathy, B., Satyanarayana, S., Khan, K. A. and Raja, K. (2017). An updated review on traditional uses, taxonomy, phytochemistry, pharmacology and toxicology of *Origanum majorana*. *International Journal of Pharma Research and Health Sciences*, 5:17-23.

Węglarz, Z., Kosakowska, O., Przybył, J. L., Pióro-Jabrucka, E. and Bączek, K. (2020). The quality of Greek oregano (*O. vulgare* L. subsp. *hirtum* (Link) Ietswaart) and common oregano (*O. vulgare* L. subsp. *vulgare*) cultivated in the temperate climate of central Europe. *Foods*, *9*(11); 1671. https://doi.org/10.3390/foods9111671